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Applicant: The Green Cross Corporation

[Title of the Invention]

Adenosine Derivatives and their Use in Cancer Immunotherapy

[Abstract]

[Object]

To provide novel adenosine derivatives with an anticancer effect based on inhibition of poly(ADP-ribose)glycohydrolase.

[Constitution]

Adenosine derivatives represented by the following general formula:

[Chemical Formula 1]

wherein R^1, R^2 and R^3 individually represent galloyl.

[Scope of Claim for a Patent]

[Claim 1]

Adenosine derivatives represented by the following general formula:

[Chemical Formula 1]

(I)

wherein R^1 represents a hydrogen atom, a group represented by the following general formula:

[Chemical Formula 2]

(II)

or A, A representing a carbonyl having a phenyl substituted by a plurality of groups selected from a group consisting of a hydroxyl group and lower-alkoxy groups, and R^2-R^6 independently represent a hydrogen atom or A, A representing the same as that described above, provided that R^1-R^3 and R^2-R^6 do not represent a hydrogen atom simultaneously.

[Claim 2]

Poly(ADP-ribose)glycohydrolase inhibitors comprising the adenosine derivatives claimed in claim 1 as effective ingredients.

[Claim 3]

Cancer immunotherapeutic agents comprising the adenosine derivatives claimed in claim 1 as effective ingredients.

[Detailed Description of the Invention]

[0001]

[Field of the Industrial Application]

The present invention relates to novel adenosine derivatives and their use.

[0002]

[Prior Art and Problems to be Solved by the Invention]

Although most of currently available anticancer agents suppress DNA synthesis or cytokinesis, they also exhibit similar effects on normal cells. Cancer therapies simply rely on utilization of a difference that cytokinesis of cancer cells is rapid, whereas that of normal cells is slow, thereby imparting more damage to cancer cells. Damage imparted to normal cells is expressed as side effect. To what degree living bodies can withstand the side effect is critical in cancer therapies.

[0003]

As is clear from the above description, cancer therapies must be essentially based on biology, biochemistry, etc., of cancer cells. Actually, however, such cancer therapies based thereon have not yet been achieved.

[0004]

Three matters, i.e., carcinogenic substances, radiation, and cancer viruses, have been pointed as causes of cancers for a long time. Among them, it was clarified that genetic information owned by cancer viruses converts normal cells into cancer cells, and thus, a term "oncogene" was created. Later, it has been hypothesized that normal cells also have oncogenes, which are switched on due to some causes, thereby converting normal cells into cancer cells. This hypothesis has been developed with time, and all those skilled in the art nowadays recognize that the hypothesis is correct on the whole.

[0005]

In the meantime, there are more than 50 types of proto-oncogenes, which can become oncogenes, in genomes of higher animals. These proto-oncogenes serve to provide significant physiological functions in the growth and differentiation of normal cells, thereby giving chances of controlling cell proliferation and cancer at a genetic level or gene products.

[0006]

An object of the present invention is to provide anticancer agents that specifically inhibit and suppress the development of oncogenes.

[0007]

It was found that the development of mouse mammary tumor virus (MMTV) gene was triggered by a de-poly(ADP-ribose) reaction in chromatin protein using mouse mammary tumor cells in which development of inserted MMTV gene was being controlled by corticoids. In other words, it is considered that the decomposition of poly(ADP-ribose) leads to local changes in chromatin structure at that region, finally resulting in binding of RNA polymerase to a promoter and promotion of transcription (Journal Biological Chemistry, 258, 15371 (1983)).

[0008]

Under such circumstance, the present inventors anticipated that when the decomposition of poly(ADP-ribose) is inhibited, oncogenes are not activated. Then, the inventors isolated and purified poly(ADP-ribose)glycohydrolase, an enzyme involving the decomposition of ADP-ribose from human placenta, and explored compounds inhibiting this enzyme. As a result, a potent inhibitory activity was found for several novel compounds. As a result of further investigation, the

inventors successfully created novel compounds that can be used as pharmaceuticals with an anticancer effect based on inhibition of poly(ADP-ribose)glycohydrolase, and thus, completed the present invention.

[0009]

[Means for Solving the Problems]

The summary of the present invention is as follows:

(1) Adenosine derivatives represented by the following general formula:

[Chemical Formula 3]

(I)

wherein R^1 represents a hydrogen atom, a group represented by the following general formula:

[Chemical Formula 4]

or A, A representing a carbonyl having a phenyl substituted by a plurality of groups selected from a

group consisting of a hydroxyl group and lower-alkoxy groups, and R^2-R^6 independently represent a hydrogen atom or A, A representing the same as that described above, provided that R^1-R^3 and R^2-R^6 do not represent a hydrogen atom simultaneously.

[0010]

(2) Poly(ADP-ribose)glycohydrolase inhibitors comprising the adenosine derivatives described in the above (1) as effective ingredients.

[0011]

(3) Cancer immunotherapeutic agents comprising the adenosine derivatives described in the above (1) as effective ingredients.

[0012]

In the present specification, lower alkoxy represented by A preferably contains one to four carbons. Specifically, methoxy, ethoxy, propoxy, iso-propoxy, butoxy, iso-butoxy, sec-butoxy, tert-butoxy, etc. are exemplified, and methoxy is particularly preferable.

[0013]

As A, those in which a phenyl is bound to a carbonyl via alkylene or alkenylene and those in which a phenyl is directly bound to a carbonyl are particularly preferred. As to alkylenes, those containing one to four carbons, such as methylene, ethylene, trimethylene,

and tetramethylene, are exemplified, and methylene and ethylene are particularly preferable. As to alkenylenes, those containing one to four carbons are exemplified and vinylene is particularly preferred.
[0014]

Preferred examples of A are groups represented by the following general formula:
[Formula 5]

wherein Z represents a direct bond, alkylene, or alkenylene, R^7-R^{11} individually represent a hydrogen atom, a hydroxyl group or a lower alkoxy, provided that R^7-R^{11} do not represent four or five hydrogen atoms simultaneously.
[0015]

Specific examples of A which are particularly preferable are galloyl, 4-hydroxy-3-methoxybenzoyl, 4-hydroxy-3,5-dimethoxybenzoyl, 3,4,5-trimethoxybenzoyl, 4-hydroxy-3-methoxycinnamoyl, 4-hydroxy-3,5-dimethoxycinnamoyl, 3,4,5-trimethoxycinnamoyl, 3,4,5-trihydroxybenzylcarbonyl and 3,4,5-trihydroxyphenetylcarbonyl.
[0016]

As a method of preparing the adenosine derivatives

(I) according to the present invention, the following method can be shown by way of example:

[Formula 6]

wherein A represents the same as that described above, $R^{1'}$ represents a hydrogen atom or is represented by the following formula:

[Chemical Formula 7]

[0017]

The above reaction takes place by an ordinary ester reaction.

[0018]

Both of compound (i) and compound (ii) as starting materials are known, and readily available. The above compound (i) is alcohol, and the compound (ii) is carboxylic acids.

[0019]

[Effects and Advantageous Results of the Invention]

The adenosine derivatives (I) according to the present invention possess a poly(ADP-ribose)glycohydrolase activity with respect to mammals including humans (humans, horses, dogs, mice, guinea pigs, rats, etc.), and are especially useful or virus infections as poly(ADP-ribose)glycohydrolase inhibitors for the treatment and prevention of malignant tumors.

[0020]

The adenosine derivatives according to the present invention are administered by themselves or in the form of pharmaceutical formulation containing the adenosine derivatives and pharmaceutically acceptable carriers. These formulations can be prepared according to methods known per se. As to dosage forms, tablets, capsules, powder, suppositories, injections, etc. are exemplified.

[0021]

The adenosine derivatives according to the present invention are administered orally and parenthetically.

[0022]

The dose of the adenosine derivatives according to the present invention can be varied according to age, weight, and severity of disease to be treated, and

response to the treatment of the patient. In case of oral administration, for example, the derivatives are administered generally at a dose of about 0.1-100 mg/kg body weight one to several times a day.

[0023]

[Examples]

Examples of the present invention will now be described in more detail, but it should be understood that the present invention is not limited to these examples.

[0024]

Example 1

Synthesis of 2',3',5'-tri-O-galloyladenosine

A solution obtained by mixing dimethylformamide (50 ml), gallic acid (10 g) and benzyl chloride (27 ml) was diluted with ethyl acetate. Then, the mixture was stirred at 140 °C overnight. The ethyl acetate layer was washed with water and a saturated saline solution, and then, dried with magnesium sulfate. After the solvent was distilled off under reduced pressure, ethanol (200 ml) and a 1.6N sodium hydroxide water solution (50 ml) were added, and the mixture was refluxed under heating for 2 hours. After reaction, about 50% of ethanol was distilled off by an evaporator. The resulting sediment was cooled to 0 °C, and then, adjusted to pH 2 with 0.5N hydrochloric acid. The

solids thus deposited were filtered off, and then, dried to obtain a compound (15.6 g, 64%). The compound (7.0 g), thionyl chloride (40 ml) and dimethylformamide (1 ml) were mixed. After the resultant solution was refluxed under heating overnight, excessive thionyl chloride was distilled off under ordinary pressure and reduced pressure to prepare 3,4,5-tribenzyloxybenzoyl chloride.

[0025]

3,4,5-tribenzyloxybenzoyl chloride (5.3 g) was added into a solution obtained by mixing adenosine (500 mg) and pyridine (10 ml). The mixture was stirred at room temperature for three days. After the reaction, the reaction solution was diluted off with ethyl acetate. The ethyl acetate layer was washed with water, 1N hydrochloric acid and a saturated saline solution, and then, dried with magnesium sulfate. The solvent was distilled off under reduced pressure, and then, was subjected to silica gel column chromatography (silica gel, solvents: ethyl acetate:hexane = 1/2, 2/3) to obtain N,N',2',3',5'-pentakis(3,4,5-tribenzyloxybenzoyl)adenosine (Intermediate Compound 1) (3.5 g, 76%).

[0026]

Intermediate Compound 1 (1.0 g), benzoylhydrazine (0.55 g) and pyridine (5 ml) were mixed. The resultant

solution was refluxed under heating for four hours. Thereafter, pyridine in the solvent was distilled off under reduced pressure. The resulting sediment was diluted with ethyl acetate. The ethyl acetate layer was washed with a saturated saline solution, and then, dried with magnesium sulfate. The solvent was distilled off under reduced pressure, and then, was subjected to silica gel column chromatography (silica gel, solvents: ethyl acetate:hexane = 1/2, 1/1, 2/1) to obtain 2',3',5'-tris-O-(3,4,5-tribenzyloxybenzoyl)adenosine (Intermediate Compound 2) (410 mg, 66%).

[0027]

After adding palladium-black (2.4 g) into Intermediate Compound 2 (2.4 g) and ethyl acetate/methanol (1/1: 100 ml), reaction was initiated under hydrogen atmosphere. After the reaction mixture was stirred at room temperature overnight, palladium-black was removed. The resulting filtrate was concentrated, and then, was subjected to liquid chromatography (column: asahipack ODP-50 preparative column, solvent: 60% methanol) to obtain a target compound, i.e., 2',3',5'-tri-O-galloyladenosine (0.75 g, 66%).

[0028]

^{1}H-NMR (DMSO-d_6)δ: 4.5-4.9 (m, 3H), 5.9-6.0 (m, 1H), 6.1-6.2 (m, 1H), 6.43 (d, J = 5.6 Hz), 6.88 (s,

2H), 6.99 (s, 2H), 7.04 (s, 2H), 7.44 (brs, 2H), 8.19 (s, 1H), 8.35 (s, 1H), 8.9-9.6 (m, 9H)

[0029]

IR (KBr, cm^{-1}): 3300, 1700, 1635, 1600

[0030]

Experimental Example 1

Poly(ADP-ribose) Glycohydrolase Inhibition Effect

^{3}H-(ADP-ribose) ?? (unreadable) was added to an assay buffer (0.01% bovine serum albumin-10 mM mercapthoethanol-50 mM potassium phosphate, pH 7.0). To 27 µl of the resulting solution, a test substance and a nuclei-derived poly(ADP-ribose)glycohydrolase solution prepared from human placenta were added to make a total volume 30 µl. The mixture was incubated at 37 °C for 1 hour. Then, the reaction mixture was absorbed by DE81 filter paper. The filter paper was washed with water, ethanol, and acetone, and then, the washings were dried. The amount of unreacted substrate ^{3}H-(ADP-ribose) was measured by a liquid scintillation counter to determine an inhibitory effect of the test substance on this enzyme. As a result, the IC_{50} value of 2',3',5'-tri-O-galloyladenosine was 30 µg/ml.

[0031]

Formulation Example 1: Tablets

(1) Compound of the present invention 10 g

(2) Fine granules for direct compression

No. 209 (Fuji Chemical)	110 g
Magnesium aluminate metasilicate	20%
Corn starch	30%
Lactose	50%
(3) Crystalline cellulose	60 g
(4) CMC calcium	18 g
(5) Magnesium stearate	2 g

[0032]

All of (1), (3), and (4) were passed through a 100-mesh sieve in advance. The (1), (3), and (4) and (2) were dried respectively to a certain water content and then, were mixed in the above weight ratio by means of a mixer. To the homogeneously mixed powder, (5) was added. The mixture thus obtained was mixed for a short time (30 seconds). The mixed powder was compressed to prepare 200 mg tablets.

[0033]

The tablets may be formulated as generally-used gastric film-coating preparations (for example, polyvinyl acetal diethyl aminoacetate) or coated with edible colorants.

[0034]

Formulation Example 2: Capsules

(1) Compound of the present invention	50 g
(2) Lactose	930 g

(3) Magnesium stearate 20 g

[0035]

The above ingredients were weighed respectively, and then, mixed homogeneously. 200 mg of the mixed powder each was filled in hard gelatin capsules.

[0036]

Formulation Example 3: Injections

(1) Compound of the present invention 5 mg

(2) Glucose 100 mg

(3) Physiological saline solution 10 ml

[0037]

The mixed solution of the above ingredients was filtered through a membrane filter, and then, was sterilized by filtration. The filtrate was fractionated aseptically to vials. After filling nitrogen gas, the vials were tightly sealed to prepare injections for intravenous administration.